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Effect of Adult Mallard Age on Avian Reproductive Tests

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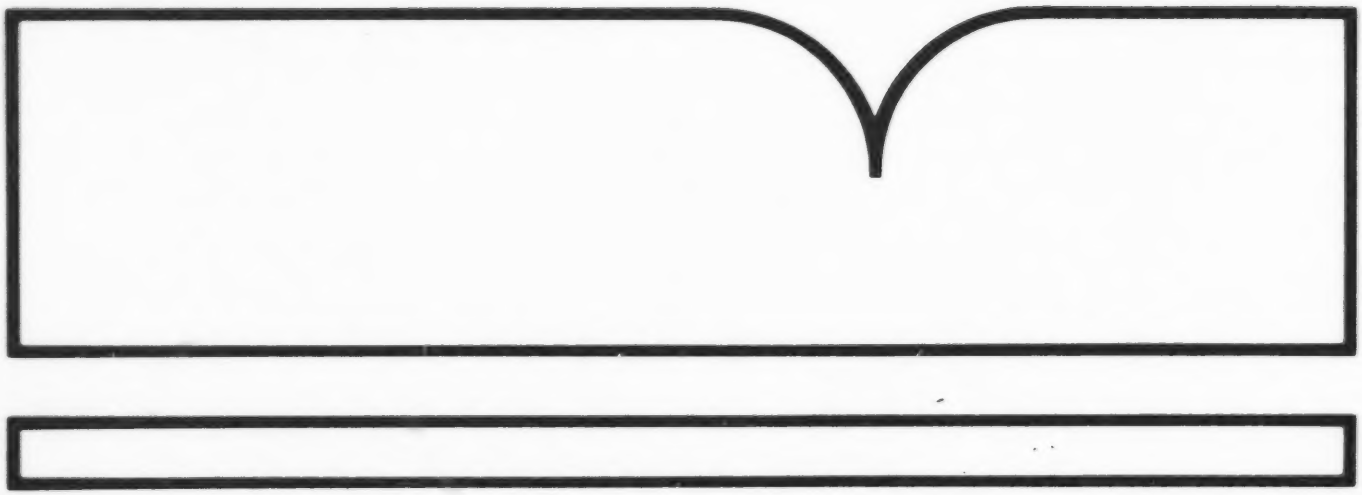

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Effect of Adult Mallard Age on Avian Reproductive Tests

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Abstract. This study was designed to determine the effect of using two different ages of mallard (*Anas platyrhynchos*) adults within the first breeding season on reproductive tests under standard Toxic Substances Control Act avian reproductive guidelines. The adult age groups were 7 and 11 months at test initiation. The test chemical was an organophosphate insecticide, chlorpyrifos. The parameters evaluated included food consumption, adult body weight, brain acetylcholinesterase levels, egg production, fertility, hatchability, egg shell thickness, egg weight, and duckling survival and weight. Chlorpyrifos exposure reduced adult body weight, brain acetylcholinesterase activity, egg production, egg shell thickness, egg weight, and day 0 duckling weight in both age groups. Statistically, adult age affected only duckling day 14 weight. However, three of the 7-month hens produced phenotypically different ducklings, suggesting the presence of a different genotype which may have impacted the day 14 weight. Overall age ranging between 7 and 11 months at test initiation did not affect this mallard reproductive test. In addition, the results of this study demonstrate the importance of using phenotypically and genotypically similar test birds.

Avian reproduction tests are included in the hazard evaluation process under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003 15 U.S.C. 2601 *et seq.*). When reproduction tests are required, they typically are conducted on both gallinaceous birds and waterfowl. Recommended test species are the bobwhite (*Colinus virginianus*) and the mallard (*Anas platyrhynchos*). Toxic Substances Control Act guidelines specify several parameters for selection of test birds: (1) the test birds should be pen reared either in the test laboratory or purchased from a commercial breeder; (2) the source should have known breeding history; (3) the source of the test birds should also be outbred with genetically wild stock to maintain the genetic heterogeneity of wild birds; (4) the test birds should be phenotypically indistinguishable from wild birds (but this does not assume identical genotypes). The guidelines further specify that the test birds be approaching their first breeding season and at least seven months old at the beginning of the test. After these guidelines had been implemented, questions arose on the potential variability of test results that might result from using young (7 month) birds. The effect of adult age (within the first breeding year) in the reproductive tests has not previously been addressed. The studies described in this paper were designed to evaluate the effect of breeder age in a standard TSCA mallard reproduction test.

Two age groups were selected at the age extremes of birds approaching their first breeding season. Birds 7 months old at test initiation represented the youngest birds that EPA would accept in a reproduction test (mallards can be forced into egg production at 25 weeks by manipulating photoperiod; Hochstein 1984). Birds 11 months old were used to represent the oldest birds which might be used in this type of study. While both age groups appeared phenotypically identical, subsequent reproductive data indicated the possibility of differing genotypes.

The organophosphate insecticide, chlorpyrifos (*O,O*-diethyl-*O* (3,5,6-trichloro-2-pyridyl) phosphorothioate) was selected as the test chemical because of its widespread use on a variety of crops and to compare with previous mallard reproductive tests conducted at the Corvallis Environmental Research Laboratory (CERL). Measured parameters included adult food consumption and body weight,

brain acetylcholinesterase, onset of laying, egg production, fertility, hatchability, shell thickness, egg weight, duckling weight, and survival.

Methods

In December 1984, 48 male and 48 female mallards (phenotypically indistinguishable from wild mallards) were purchased from Whistling Wings Inc. (Hanover, IL).[1] One-half of each sex was 7 months old at the start of the test and the other half was 11 months old. All birds were leg-banded and wing-clipped. A licensed veterinarian examined a random sample of each age group for general health and possible parasitic infection; all birds examined were healthy. Twenty-four pairs from each age group were randomly assigned to reproductive pens (0.8 m × 1.6 × 1.0 H front, 0.7 m back, 1 m off floor) housed in a plastic covered greenhouse (33.5 m x 6.1 m X 3.6 m high). The light cycle and intensity were controlled by ambient conditions. The greenhouse is equipped with a 60,000 BTU propane heater which maintained the interior temperature above freezing in the winter and an evaporative cooling system to keep the temperature below 32°C during the summer. Water supply to the greenhouse was untreated well water. Each pen contained a flow-through water system, individual feeder, nest box, and was visually separated from adjacent pens by plastic partitions.

One bird produced an egg during shipment. Thus, the birds were initially fed a maintenance diet (to delay egg production) amended with 2% corn oil. The birds were weighed initially and allowed to acclimate for two weeks (receiving the corn oil amended maintenance diet). They were reweighed and placed on the test diet, either maintenance feed plus 2% corn oil only or maintenance feed, 2% corn oil, and technical grade (96% pure) chlorpyrifos to produce a treatment level of 80 ppm ± 10% (confirmed by gas chromatography analysis). This concentration of chlorpyrifos was selected because it is approximately 10% of the LC_{50} value (LC_{50} = 800 mg/kg for 14-day-old birds; Gile *et al.* 1983). Feed was provided *ad libitum* and measured weekly to determine consumption. The birds were weighed at 2-week intervals until the onset of laying and then at the end of the study (12 weeks after laying began). The diet was changed to a standard layer feed with the appropriate corn oil and/or chemical ammendment after the birds began to lay (approximately 6 weeks after starting test diet). Although the standard TSCA protocol specifies a 10-week prelaying exposure, the natural light conditions in the greenhouse resulted in the onset of egg production in some hens after only 6 weeks of exposure to the test diet. Egg collection commenced at this point. The 10-week pre-laying period was designed primarily for chemicals that bioaccumulate and therefore might have an increased effect. There is no evidence that chlorpyrifos bioaccumulates (Kenaga 1974).

Eggs were collected daily, marked with date and pen number, and stored at 12.5°C ± 2°C on turning trays for no more than 7 days. Eggs were set weekly in a Humidaire Model 150 incubator. All eggs were candled on day 7 to determine fertility and then not handled until transfer to a Humidaire Model D hatcher on day 24. Between day 7 and day 24 all eggs received a daily light misting with water from a plant sprayer to improve hatchability. A local duck breeder reported a consistent 5–8% hatch increase as a result of the misting practice (personal communication, David Holderread 1985). An additional Georgia Quail Farm (GQF) incubator was used during peak production period for the first 7 days incubation of each egg hatch. The median hatch day was day 27; all unhatched eggs were discarded on day 29. Upon hatching, each duckling received a wing tag providing individual identity and pen or parent identity. The ducklings were weighed on day zero and placed in standard GQF duck brooders for 14 days, then euthanized with CO_2 and reweighed. During that 2-week observation period, the hatchlings received a standard game bird starter chow and water *ad libitum*.

Exposure to organophosphate insecticides such as chlorpyrifos inhibits brain acetylcholinesterase activity (Hill and Camardese 1984). Acetylcholinesterase assays were performed with a modified Ellman technique (Ellman *et al.* 1961) on a Perkin-Elmer Lambda/UV-VIS spectrophotometer. Analysis of variance (ANOVA) was performed on food consumption, acetylcholinesterase activity, duckling weight, and reproductive data (Statistical Analysis System 1982). An arcsin transformation was performed on percent fertility and percent hatchability data prior to the analysis of variance test (Sokal and Rohlf 1981). The α = 0.05 significant level was selected for statistical tests unless otherwise indicated.

Results

The adult birds consumed equivalent amounts of food irrespective of age or treatment (Table 1). Birds of both age groups receiving the treated diet consumed a maximum of 1.2 g of chlorpyrifos over the length of the study (as calculated from total food consumption). Food consumption varied significantly over time (based on paired Student's t-test; Sokal and Rohlf 1979). Figure 1 depicts the pattern of food consumption for all groups; the onset of laying at week 7 triggered a dramatic increase in food consumption.

While there was no significant difference in overall food consumption between the four test groups, exposure to chlorpyrifos resulted in a significant weight loss for both males and females of both ages when compared to the controls (Table 1, Figure 2). All birds gained weight during the 14-day pre-test acclimation period. Treated males showed a significant (α = 0.05) decline in body weight during the test. Control females of both ages showed a significant weight gain coinciding with egg production, whereas the treated females continued to lose weight. Necropsy results indicated that only one hen (7 month, 80 ppm) was not reproductively active at the termination of the study.

Exposure to chlorpyrifos resulted in reduced brain acetylcholinesterase activity (Table 2). All treated birds of both sexes had significantly lower acetylcholinesterase activity when compared to controls. Treated females were significantly lower than treated males. The acetylcholinesterase levels were not affected by age of the birds.

[1] Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Table 1. Food consumption and body weight changes for adult mallards

	Food Consumption, g/b/d[a]			
	7-Month adults		11-Month adults	
	Control	80 ppm	Control	80 ppm
	120.70 (7.1)[b]	116.50 (9.8)	135.1 (11.5)	117.2 (12.6)
	Net body weight change[c] (%)			
Male	−8.7 (2.6)	−17.4 (2.6)*	−11.7 (1.9)	−16.1 (2.2)*
Female	+18.5 (3.3)	−19.0 (3.4)*	+9.9 (3.6)	−16.8 (2.9)*

[a] Mean grams per bird per day
[b] Values in parentheses represent standard error = standard deviation / $\sqrt{N}$
[c] Weight change during exposure to chlorpyrifos
* Significantly different change in body weight during chlorpyrifos exposure at the 0.05 level

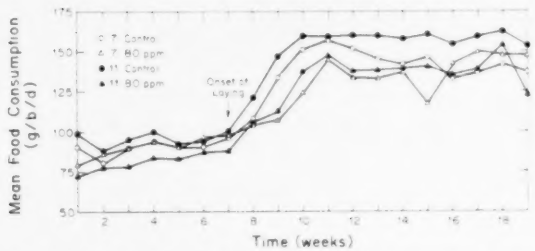

Fig. 1. Adult mallard food consumption profile (note: only significant effect was due to onset of egg production)

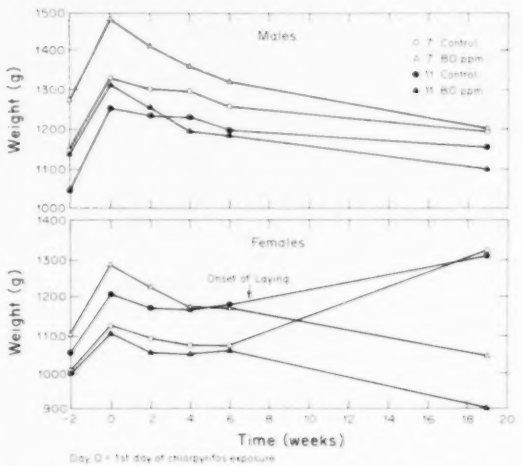

Fig. 2. Adult mallard weight profile (note: only significant effect ($\alpha = 0.05$) was an increased weight loss among both chlorpyrifos-treated males and females

Table 3 summarizes egg production data. The onset of laying was not significantly different for any group. Total egg production, expressed as eggs/hen-day was not affected by the age of breeders. Treated birds of both ages, however, produced significantly fewer eggs which weighed less and had thinner shells than controls. Neither age nor chlorpyrifos exposure affected fertility or hatchability. Figure 3 profiles egg production. The egg production plateau for the treated birds of both ages was not only lower than controls but also lagged behind by approximately one week. Once peak production was reached, it remained relatively constant throughout the study.

Duckling survival to day 14 was identical between test groups (Table 4). Duckling weight presented a confounding picture. At day 0, ducklings from treated adults of both ages were significantly smaller, corresponding to the smaller eggs noted in Table 3. By day 14, the ducklings from the 7-month-old adults were significantly larger than those from the 11-month old adults, irrespective of treatment.

Three of the 7-month old hens produced several ducklings that were phenotypically different. These ducklings were completely yellow in color rather than the typical black and yellow mallard ducklings. The weights of these birds at day 14 were generally greater than the other ducklings. Several of these ducklings were maintained after the 14 day observation period and allowed to achieve adult plumage. As adults, they were completely white and sexed a males. The day 14 weight data was analyzed with and without these ducklings. In both cases, the day 14 weight of the ducklings from the 7-month old breeders was significantly greater than that of the ducklings produced by the 11-month old breeders.

Discussion and Conclusions

These results confirm the effect of chlorpyrifos on mallard reproduction (Gile 1984). In both studies, the amounts of food consumed and the effects of

Table 2. Adult brain acetylcholinesterase[a]

	7-Month adults			11-Month adults		
	Control	80 ppm	% of Control	Control	80 ppm	% of Control
Male	2.47 (0.07)[b]	1.64 (0.10)*,**	59	2.57 (0.03)	1.52 (0.09)*,**	59
Female	2.57 (0.04)	1.29 (0.06)*	50	2.56 (0.02)	1.25 (0.09)*	49

[a] μMoles acetylthiocholine iodide hydrolyzed/mm/g tissue
[b] Values in parentheses represent standard error = standard deviation $\sqrt{N}$
* Significantly different from control values at the 0.05 level
** Significantly different from treated females at the 0.05 level

Table 3. Egg production

	7-Month adults		11-Month adults	
	Control	80 ppm	Control	80 ppm
Onset of laying[a]	59.7 (1.9)[b]	62.5 (3.4)	57.5 (2.2)	63.9 (2.7)
$\bar{x}$ No. eggs/hen during entire study	54.3 (4.9)	36.3 (6.3)	61.4 (4.5)	36.1 (4.1)
No. Hen-days[c]	1716	1652	1716	1700
Eggs/Hen-day[d]	0.38 (0.03)	0.26 (0.04)*	0.43 (0.03)	0.25 (0.03)*
% Fertility[e]	94.8 (3.7)	94.0 (2.1)	89.0 (4.2)	89.9 (3.1)
% Hatchability[f]	72.1 (4.1)	65.2 (5.8)	56.0 (6.2)	56.7 (7.8)
$\bar{x}$ Shell thickness (mm)	0.405 (0.007)	0.364 (0.009)*	0.394 (0.006)	0.372 (0.005)*
$\bar{x}$ Egg weight (g)	63.31 (1.40)	59.21 (1.69)*	64.24 (1.23)	57.58 (1.47)*

[a] Number of days after initiation of test
[b] Values in parentheses represent standard error = standard deviation / $\sqrt{N}$
[c] Total number of days available for egg production
[d] Total eggs produced by group/hen-day
[e] No. of eggs surviving to day 7 of incubation/no. of eggs set
[f] No. hatched/no. fertile
* Significantly different from control values at the 0.05 level

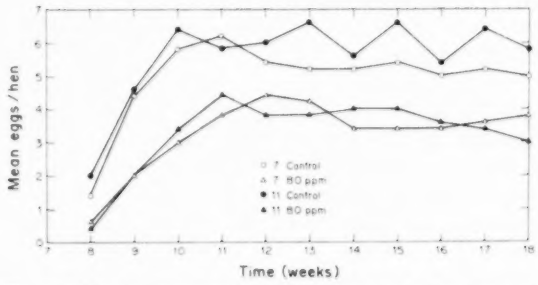

Fig. 3. Mallard egg production profile (note: only significant effect due to chlorpyrifos exposure)

chlorpyrifos on adult weight, brain acetylcholinesterase, and egg production were similar. Food consumption for controls and 80 ppm treated birds in the previous study was approximately 130 g/b/d compared to 123 g/b/d for this study. Control males in the previous study maintained their initial weight, while control females exhibited a significant weight gain. Treated males and females both experienced significant weight loss. Acetylcholinesterase levels for the treated males and females were 43% lower than controls in the previous study. The average difference in acetylcholinesterase activity between treated and controls in this study was again 43%. Treated birds from both studies produced 35% fewer eggs. As in this study, the egg weights from the treated birds in the previous study (Gile 1984) were also smaller. These data suggest that the chemical effects are consistent.

While the effect of breeder age on reproductive ability ages within the first breeding season has not been addressed in the literature, several studies have examined mallard reproductive parameters in general (Hochstein 1984; Batt and Prince 1978; Hazeltine and Henry, in press; Piccirillo and Quesenberry 1980). The reproductive data fall within the range of values observed by these investigators.

This study examined 12 parameters relating directly or indirectly to reproductive capacity (adult

Table 4. Duckling survival and weight

	7-Month adults		11-Month adults	
	Control	80 ppm	Control	80 ppm
Survival (%(	99	98	97	98
$\bar{x}$ Day 0 Wt. (g)	41.8 (0.702)[a]	37.8 (1.06)*	41.3 (0.723)	37.5 (0.598)*
$\bar{x}$ Day 14 Wt. (g)	298.0 (4.81)	305.0 (6.95)	280.0 (6.17)**	269.0 (3.46)**

[a] Values in parentheses represent standard error = standard deviation / $\sqrt{N}$
* Significantly different from control values at 0.05 level
** Significantly different from 7 month groups at 0.05 level

food consumption, adult weight, brain acetylcholinesterase activity, onset of laying, egg production, fertility, hatchability, shell thickness, egg weight, duckling weights on day 0 and 14, and duckling survival). Duckling day 14 weight was the only measured parameter which was affected by adult age. As previously noted, three of the 7-month old hens produced atypically colored ducklings indicating a genotype different from the typical mallard. It is possible that this phenotypical difference, although not evident in the offspring from all 7-month old hens, was not just restricted to the three pens identified by the atypical ducklings. While all of the adult birds were purchased from the same commercial source, it is possible that the parental gene pool producing the 7-month old adults differed from that of the 11-month old test birds. These atypical ducklings were consistently larger at day 14 than the other ducklings. Even though statistical testing indicated an apparent effect of adult age upon duckling growth, this may actually be the result of a potentially different gene pool of the 7-month adults.

This study evaluated the effect of adult age in mallard reproductive tests. Since 11 of the 12 parameters tested were not affected by age, it is concluded that breeder age was probably not a factor in this test. No test was made for the effect of differing genotypes on reproduction. However, the results of what may be a difference in adult genotype amplifies the need to be cautious when selecting test mallards. It is important to remember that these birds are representatives of a very heterogeneous population and are not like the "laboratory rat." Currently, TSCA guidelines consider breeding history to include lighting, disease record, drug administration, and age. Of equal importance may be the genetic history of the colony.

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